

Multi-data set revision of two uncommon species of Chromodorididae (Nudibranchia) from the Gulf of Mexico

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Abstract: Morphological and molecular data are used to address the taxonomic status of two uncommon species of opisthobranch molluscs from the Gulf of Mexico. *Chromodoris fentoni* is a new species closely related to other Atlantic and eastern Pacific congeneric species. It is characterized by having a whitish background color almost completely covered with irregular red pigment. *Glossodoris punctilucens* Bergh, 1890 is a rare species known from only a handful of specimens. Morphological and molecular data confirm that it is distinct from other similarly colored species from the eastern Atlantic.

Key words: *Chromodoris*, *Glossodoris*, morphology, COI, H3

Several species of Chromodorididae have been described from the Caribbean and Gulf of Mexico. Most of the historic descriptions are based on preserved material and therefore lack information on the color of the living animals (Valdés *et al.* 2006). Modern publications generally include both descriptions of the living animals and anatomical data (Valdés *et al.* 2006), greatly improving the possibility of other authors identifying these species. However, it has become evident that color and anatomical information alone are not always sufficient to provide a solid framework for solving complex taxonomic issues involving closely related species. Pola *et al.* (2006) illustrated how molecular information combined with anatomical data can be used to effectively address problems of color variation and species boundaries in an eastern Pacific opisthobranch species complex, opening the door for applying this methodology to other groups of species.

In this paper, we attempt to clarify the systematics of two uncommon species using a combination of external morphology, anatomy, biology, and sequence data from a mitochondrial and a nuclear gene, and comparing this information with other closely related species.

MATERIALS AND METHODS

Collection and preservation

The specimens were collected from the Gulf of Mexico, west Florida shelf. This area consists of a hard-bottom habitat of low relief carbonate structures, commonly called reef ledges. These discontinuous ledges are generally oriented north-south, rise from less than one meter to several meters

off the sandy bottom, and support a diverse community of marine organisms. On 30 March 2009, while scuba diving at a depth of 9 m, commercial aquarium trade fisher, Daniel Fenton, collected by hand a specimen of the red sponge *Igeruella notabilis*, which contained two specimens of *Chromodoris fentoni*. The sponge and nudibranchs were placed into plastic bags with seawater and transported to the Florida Fish and Wildlife Conservation Commission's (FWC), Fish and Wildlife Research Institute (FWRI). Features of these living organisms were digitally photographed in a photographic plexiglass aquarium at the FWRI.

On two subsequent field trips with Daniel Fenton, FWRI scientific research scuba divers, at a depth of 9-10 m, searched along ledges for *Igernella notabilis* where *Chromodoris fentoni* had been previously collected. While conducting the search for *C. fentoni* on 21 June 2009, *Glossodoris punctilucens* Bergh, 1890 was found traversing the substrate. On 21 July 2009, *C. fentoni* was found on *I. notabilis*. On each occasion, FWRI scientific divers digitally photographed the specimens *in situ* and then collected them by hand, placed them into a plastic bag while underwater, and then transferred them from the bag into a five gallon bucket of seawater for transport back to the FWRI. While at the FWRI, the specimens were digitally photographed in a photographic plexiglass aquarium. During subsequent field trips by D. Fenton more specimens of *G. punctilucens* were collected: on 28 January 2010 two specimens were found on an unidentified sponge and on 3 June 2010 two more specimens were collected on the same species of sponge. This time the sponge was collected and tentatively identified as *Ircinia* cf. *campana*. On a separate occasion D. Fenton (pers. comm.) observed *G. punctilucens* feeding on

this sponge species and the nudibranchs left feeding markings as they moved on the surface of the sponge. All collected specimens were fixed and preserved in 95% ethanol to facilitate genetic analyses. All specimens were catalogued and accessioned, and were deposited in the FWRI Specimen Information Services (SIS) collection of the Florida Fish and Wildlife Conservation Commission - Fish and Wildlife Research Institute (abbreviated as FSBC).

Morphological examination

Preserved specimens were dissected and the internal features were examined and drawn using a dissecting microscope (Nikon SMZ-100) with the aid of a *camera lucida* attachment. The buccal mass of one individual of each species was removed and dissolved in 10% sodium hydroxide until the radula and jaw were isolated from the surrounding tissue.

The radula and jaw were then rinsed in water, dried, mounted, and sputter coated for examination with a scanning electron microscope (SEM) Hitachi S-3000N at the Natural History Museum of Los Angeles County.

DNA extraction

In addition to the specimens collected for this study, several other species were sequenced because of their color similarity with *Chromodoris fentoni*. These include *Chromodoris rolani* Ortea, 1988, *Chromodoris sphoni* (Ev. Marcus, 1971), and *Glossodoris baumanni* (Bertsch, 1970) (Table 1). DNA extraction was performed using either a hot Chelex[®] protocol or the DNeasy Blood and Tissue Kit (Qiagen). Approximately 1-3 mg of the foot was cut into fine pieces for extraction for both protocols. For the Chelex extraction, the foot tissue was rinsed and rehydrated using

Table 1. List of specimens used in the molecular study, including locality, voucher number, and GenBank Accession numbers. Abbreviations: FSBC, Florida Fish and Wildlife Conservation Commission - Fish and Wildlife Research Institute; SAM, South Australian Museum, Adelaide; LACM, Natural History Museum of Los Angeles County.

Species	Locality	Voucher No.	GenBank Accession No.	
			H3	COI
<i>Chromodoris alternata</i>	-	SAM D19281	-	EF535120
<i>C. ambiguus</i>	-	SAM D19260	-	EF535119
<i>C. epicuria</i>	-	SAM D19285	-	EF535114
<i>C. fentoni</i>	Pinellas County, FL	FSBC I 67095	GU815542	GU815540
<i>C. krohni</i>	-	-	-	AF249805
<i>C. krohni</i>	-	-	-	AY345036
<i>C. kuiteri</i>	-	-	-	AF249804
<i>C. kuniei</i>	-	SAM D19261	-	EF535112
<i>C. leopardus</i>	-	SAM D19288	-	EF535116
<i>C. luteorosea</i>	-	-	-	AJ223259
<i>C. magnifica</i>	-	SAM D19290	-	EF535110
<i>C. purpurea</i>	-	-	-	AJ223260
<i>C. quadricolor</i>	-	-	-	AF249802
<i>C. rolani</i>	Cape Verde	LACM 153124	GU815543	GU815541
<i>C. sphoni</i>	Huatulco, Mexico	-	GU815544	-
<i>C. sphoni</i>	-	LACM 175026	GU815545	-
<i>C. splendida</i>	-	SAM D19292	-	EF535115
<i>C. striatella</i>	-	SAM D19293	-	EF535111
<i>C. tasmaniensis</i>	-	SAM D19295	-	EF535113
<i>Glossodoris atromarginata</i>	-	-	-	AF249789
<i>G. baumanni</i>	Huatulco, Mexico	-	GU815546	-
<i>G. cincta</i>	-	-	-	EF535136
<i>G. edmundsi</i>	-	-	-	EF535133
<i>G. pallida</i>	-	-	-	EF535138
<i>G. pullata</i>	-	-	-	EF535137
<i>G. punctilucens</i>	Pinellas County, FL	FSBC I 67201	GU815547	GU815539
<i>G. sedna</i>	-	-	-	EF535134
<i>G. sibogae</i>	-	-	-	EF535135
<i>Noumea haliclona</i>	-	SAM D19269	-	EF535117

1.0 mL TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) for 20 minutes. A 10% (w/v) Chelex 100 (100-200 mesh, sodium form, Bio-Rad) was prepared using TE buffer. After re-hydration, the mixture was then centrifuged, 975.00 μ L of the supernatant was removed, and 175.00 μ L of the Chelex solution was added. Samples were then heated in a 56 °C water bath for 20 minutes, heated in a 100 °C heating block for 8 minutes, and the supernatant was used for PCR. The DNeasy protocol supplied by the manufacturer was followed, with some modifications. The elution step was modified such that the first elution was collected using 100.00 μ L of Buffer AE and was allowed to incubate at room temperature for 5 minutes. In a new test tube, a second elution step was conducted using 200.00 μ L of Buffer AE and was also allowed to incubate at room temperature for 5 minutes. The first elution was used for PCR.

PCR amplification and sequencing

Colgan's universal H3 primers (Colgan *et al.* 1998) were used with all specimens to amplify the region of interest. Folmer's universal COI primers (Folmer *et al.* 1994) were used to amplify the regions of interest for all specimens (Table 1).

The master mix was prepared using 34.75 μ L H₂O, 5.00 μ L Buffer B (ExACTGene, Fisher Scientific), 5.00 μ L 25 mM MgCl₂, 1.00 μ L 40mM dNTPs, 1.00 μ L 10mM primer 1, 1.00 μ L primer 2, 0.25 μ L 5 mg/mL Taq, and 2.00 μ L extracted DNA. Reaction conditions for H3 were as follows: an initial denaturation for 2 min at 94 °C, 35 cycles of (1) denaturation for 30 sec at 94 °C, (2) annealing for 30 sec at 50 °C, and (3) elongation for 1 min at 72 °C, and a final elongation for 7 min at 72 °C. Reaction conditions for COI involved an initial denaturation for 2 min at 94 °C, 35 cycles of (1) denaturation for

30 sec at 94 °C, (2) annealing for 30 sec at 50 °C, and (3) elongation for 1 min at 72 °C, and a final elongation for 7 min at 72 °C.

PCR products yielding bands of appropriate size (approx. 375 bp for H3 and 700 bp for COI) were purified using the Montage PCR Cleanup Kit (Millipore). Cleaned PCR samples were quantified using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). Each primer was diluted to 2.0 pmol/ μ L to send out for sequencing with the PCR products. PCR products were diluted to 6.0, 7.5, and 11.5 ng/ μ L for H3 and COI, respectively. Samples were sequenced at the City of Hope DNA Sequencing Laboratory (Duarte, California) using chemistry types BigDye V1.1 for fragments less than 500 bp and BigDye V3.1 for fragments larger than 500 bp.

Phylogenetic analyses

Sequences for each gene were assembled and edited using Geneious Pro 4.7.4 (Biomatters Ltd.). Geneious was also used to extract the consensus sequence and to construct the alignment for each gene using the default parameters. The sequences were not trimmed after alignment. A total of 328 bp for H3 and 658 bp for COI were used for the phylogenetic analyses. Analyses were conducted for species of *Chromodoris* and *Glossodoris* separately and together, using COI data and including sequences available in GenBank (Table 1). Additionally, an analysis of all the H3 available sequences was conducted. For the *Glossodoris* COI analysis, *Chromodoris fentoni* was selected as the outgroup and for the *Chromodoris* COI and H3 analyses, *Glossodoris punctilucens* was selected as the outgroup. For the combined *Glossodoris* + *Chromodoris* COI analysis, *Noumea haliclona* (Burn, 1957) was selected as the outgroup.

The levels of saturation for each gene and for the first and second versus third codon positions of COI and H3 were

Table 2. Summary of each data set used for analysis with the best-fit evolutionary models and estimated parameters.

Parameters	H3	<i>Chromodoris</i> COI	<i>Glossodoris</i> COI	<i>Chromodoris</i> + <i>Glossodoris</i> COI
No. of specimens used	6	18	9	25
No. of included characters	328	587	587	587
Best-fit model	TrN+I	GTR+I+G	TIM2+I+G	GTR+I+G
Frequency A	0.2468	0.3046	0.2511	0.3080
Frequency C	0.3041	0.0945	0.1108	0.0857
Frequency G	0.2545	0.1606	0.1820	0.1621
Frequency T	0.1946	0.4403	0.4560	0.4441
R-matrix [A-C]	1.0000	1.6928	0.0730	1.5579
R-matrix [A-G]	5.4423	23.4219	9.8678	19.6718
R-matrix [A-T]	1.0000	1.0448	0.0730	0.6047
R-matrix [C-G]	1.0000	11.8056	1.0000	11.3414
R-matrix [C-T]	13.0475	166.4768	76.9023	170.8606
R-matrix [G-T]	1.0000	1.0000	1.0000	1.0000
Γ shape (G)	-	0.5470	0.1850	0.2680
Proportion of Invariant Sites (I)	0.7840	0.5660	0.4240	0.4607

investigated using the substitution saturation test developed by Xia *et al.* (2003) and Xia and Lemey (2009) implemented in the program DAMBE (Xia and Xie 2001).

The Akaike information criterion (Akaike 1974) was executed in jModelTest (Posada 2008) to determine the best-fit model of evolution (Table 2). Maximum likelihood analyses were conducted using PAUP*4.0 (Swofford 2002). Robustness of each clade was assessed by bootstrap support (Felsenstein 1985) based on 2000 replicates with heuristic search, TBR branch-swapping algorithm, multrees option, 100 random additions. For the combined *Chromodoris* + *Glossodoris* COI analysis only 1 random addition for each bootstrap replicate was investigated due to computer power constraints.

SPECIES DESCRIPTIONS

Chromodoris fentoni new species
(Figs. 1A-B, 2-3)

Material examined

Holotype: off Pinellas County, Florida (28.17100°N, 83.00200°W), 30 March 2009, 9 m depth, specimen 15 mm preserved length, leg. D. Fenton (FSBC I 67094). Paratypes: off Pinellas County, Florida (28.17100°N, 83.00200°W), 30 March 2009, 9 m depth, 1 specimen 16 mm preserved length, dissected, leg. D. Fenton (FSBC I 67095). Off Pinellas County, Florida (28.07076°N, 82.54663°W), 21 July 2009, 9 m depth, 1 specimen 8 mm long, leg. N. Sheridan (FSBC I 67253).

External morphology

The body has a whitish background color almost completely covered with irregular red pigment (giving the animal a reddish appearance) and many yellow spots with an orange center (Fig. 1A). The yellow-orange spots are more densely arranged on the mantle margin. The mantle edge is surrounded by a translucent grayish band, followed by a whitish band, and a narrow yellow line. The branchial leaves (Fig. 1B) and rhinophores are white with a few reddish spots and a submarginal reddish patch on each leaf and rhinophore; the apices of the leaves and rhinophores are opaque white.

There are 10 branchial leaves surrounding the anus. The mantle margin contains two disorganized bands of small, discrete, oval mantle glands.

Reproductive system

The reproductive system contains a long, narrow ampulla that connects to the prostate and the female gland complex (Fig. 2B). The prostate is long and convoluted and narrows into a long, muscular deferent duct that expands into the broad muscular portion of the deferent duct (Fig. 2A). The vagina is short, slightly convoluted and connects directly into the large, irregular bursa copulatrix. The seminal receptacle is elongate, and also connects directly into the bursa copulatrix at some distance from the vaginal insertion. The straight uterine duct leaves the bursa copulatrix next to the insertion of the seminal receptacle and inserts into the female gland complex, near the opening of the ampulla.

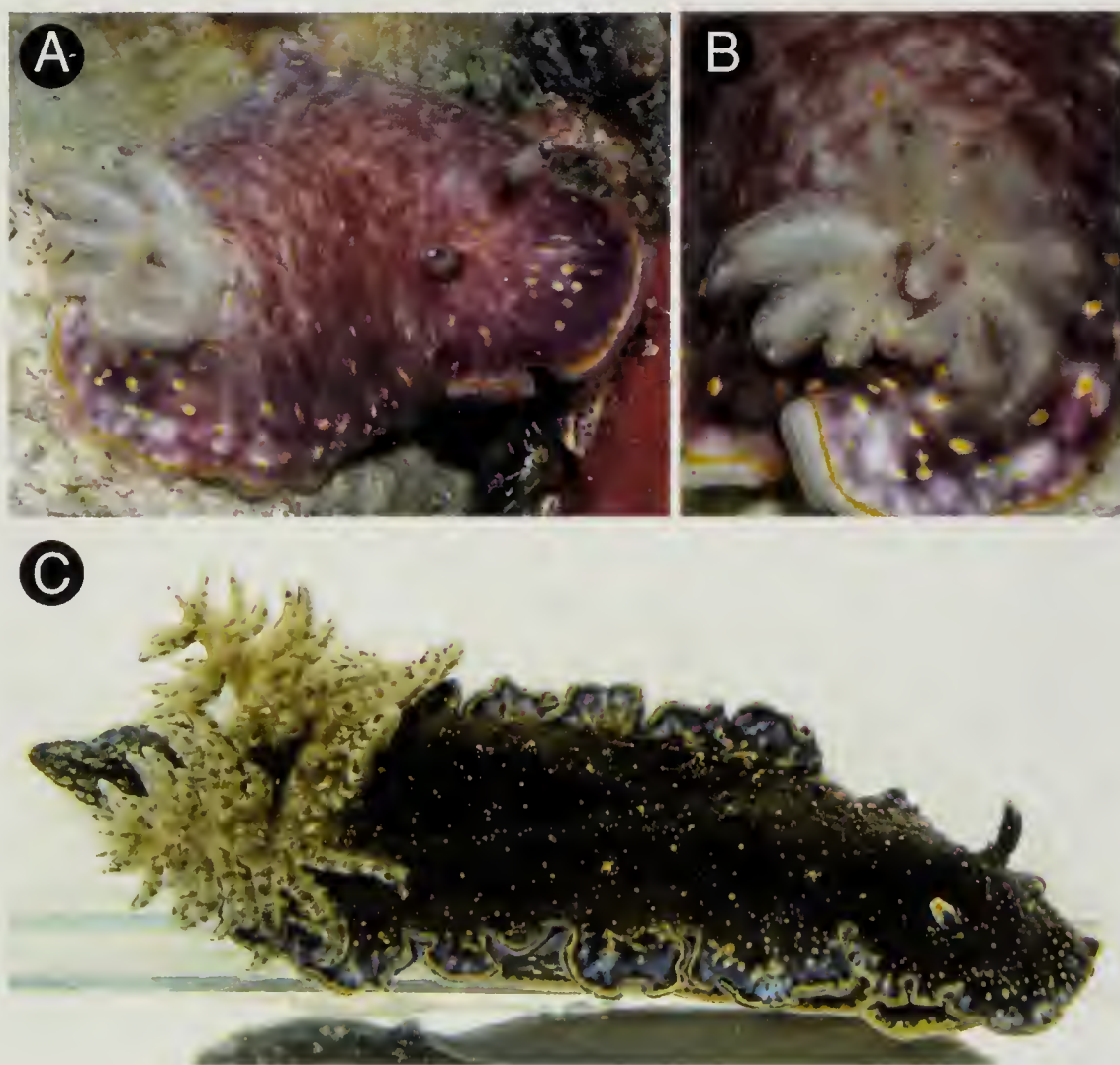


Figure 1. Photographs of living animals. A, Aquarium photograph of the holotype of *Chromodoris fentoni* (FSBC I 67094) by M. Colella; B, Detail of the gill of the holotype of *Chromodoris fentoni* (FSBC I 67094) by M. Colella; C, Aquarium photograph of *Glossodoris punctiluceus* (FSBC I 67201) by N. Sheridan.

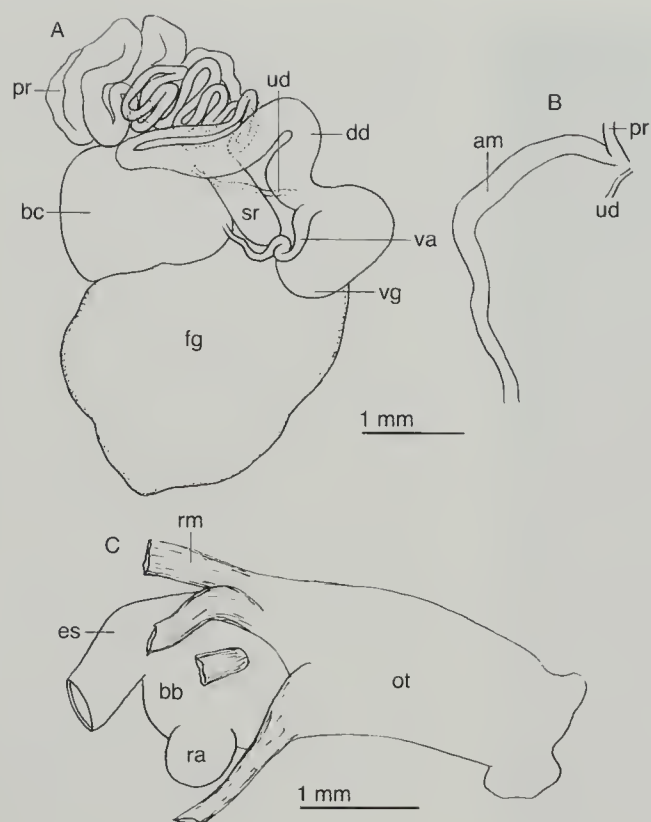


Figure 2. Drawings of the anatomy of the paratype of *Chromodoris fentoni* (FSBC I 67095). A, Reproductive system; B, Detail of the ampulla; C, Anterior portion of the digestive system. Abbreviations: am, ampulla; bb, buccal bulb; bc, bursa copulatrix; dd, deferent duct; es, esophagus; fg, female gland complex; ot, oral tube; pr, prostate; ra, radular sac; rm, retractor muscle; sr, seminal receptacle; ud, uterine duct; va, vagina; vg, vestibular gland.

Digestive system

The buccal bulb is relatively short, about three times shorter than the oral tube (Fig. 2C). The radular formula is $54 \times 23.0.23$ in the paratype. There are no rachidian teeth. The innermost lateral teeth are wider than the rest of the laterals (Fig. 3A), and have an elongate cusp with 1-2 inner denticles and 3-4 outer denticles. The mid lateral teeth are hook-shaped with 6-8 denticles (Fig. 3C). The outer laterals have 3-6 denticles (Fig. 3B). The jaw consists of numerous bicuspid rodlets (Fig. 3D).

Molecular data

Chromodoris fentoni is genetically distinct from other species of *Chromodoris* for which we have molecular data. The data sets available are too incomplete to say anything relevant about the phylogenetic relationships of this species but allow us to compare it with other morphologically closely related species. For example, the most similar species in regard to the external coloration are the eastern Pacific *Glossodoris baumanni* (Bertsch, 1970) and *Chromodoris sphoni* (Ev. Marcus, 1971), which show sequence divergences of 4.8% and 2.4%, respectively, with *C. fentoni* in the H3 gene.

Biology

All specimens were collected on the red sponge *Igernella notabilis* (Demospongiae: Ceractinomorpha: Dendroceratida: Dictyodendrillidae), at 9 m depth, which most likely constitutes their diet. The sponge specimen is deposited at the FWRI collections (FSBC I 67300). This species is known only from the Gulf of Mexico.

Etymology

The species is named after Daniel Fenton (Florida), the collector of the original type specimens.

Remarks

The generic placement of *Chromodoris fentoni* is problematic as this species displays characteristics of both *Chromodoris* and *Glossodoris* according to the diagnoses by Rudman (1984). For instance, the double row of mantle glands and the long oral tube in comparison to the buccal bulb are characteristics of members of *Glossodoris* (Rudman 1984). However, the radula of *C. fentoni* is relatively short and wide, protruding as a short radular sac, the body profile is relatively low and wide, the gill is composed of leaves arranged in a semi-circle. All these characteristics are consistent with Rudman's (1984) diagnosis of *Chromodoris* and the features found in other western Atlantic species of this group. More importantly, molecular data reveal that *C. fentoni* is more closely related to *Chromodoris krohni* (Vérany, 1946) than to any species of *Glossodoris*.

Chromodoris fentoni is clearly distinguishable from other Atlantic and Eastern Pacific species of *Chromodoris*. The most similar species in external coloration is the Eastern Pacific *Glossodoris baumanni* (Bertsch, 1970), which also has a light background color with irregular red pigment, and whitish rhinophores and gills with subapical reddish pigment (Gosliner *et al.* 2004). However, *G. baumanni* lacks the characteristic yellow-orange spots present in *C. fentoni*. Anatomically, these two species are very different as *G. baumanni* has very elongate mid-lateral teeth with the denticles concentrated near the end of the cusp (Gosliner *et al.* 2004), whereas in *C. fentoni* the mid-lateral teeth are shorter and the denticles more evenly distributed. In *G. baumanni* the rachidian row of teeth may be present or absent. The reproductive system of *G. baumanni* has a highly developed deferent duct and the uterine duct is partially fused with the vagina (Gosliner *et al.* 2004), both characteristics are absent in *C. fentoni*. Incidentally, Gosliner *et al.* (2004) transferred *G. baumanni* to the genus *Glossodoris*, but preliminary molecular data here presented seem to suggest that the original placement in *Chromodoris* (Bertsch 1970) was correct although support levels are too low to reach a definitive conclusion.

Another Eastern Pacific species, *Chromodoris sphoni* (Ev. Marcus, 1971), has similar rhinophore and gill coloration to

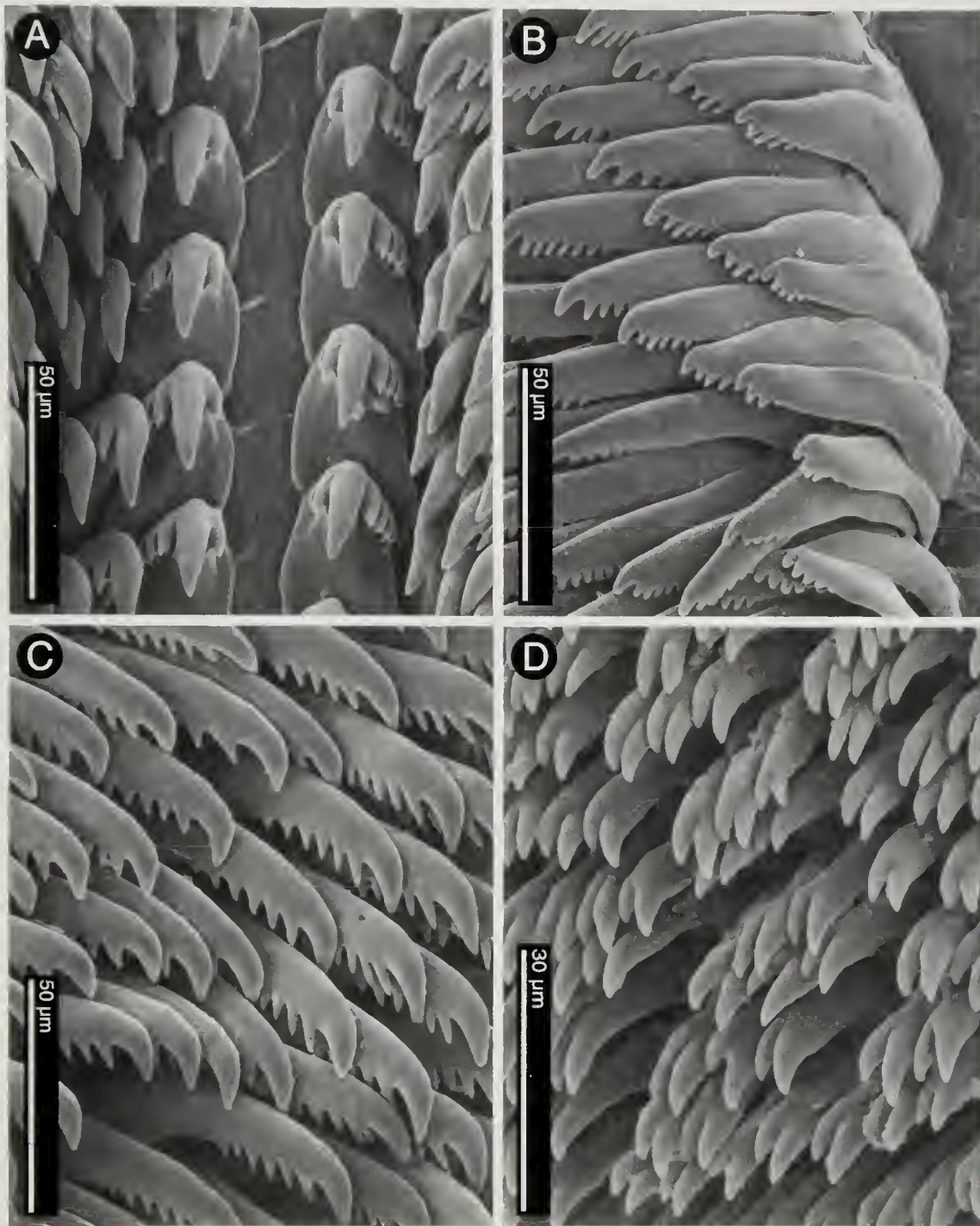


Figure 3. SEM micrographs of the radula and jaws of the paratype of *Chromodoris fentoni* (FSBC I 67095). A, Innermost lateral radular teeth; B, Outermost lateral radular teeth; C, Mid-lateral radular teeth; D, Jaw rodlets.

C. fentoni, and also has dorsal yellow spots, but the color pattern is very different, having a bluish background color with a solid red cross pattern on the dorsum (Ortea *et al.* 1992). The anatomy of *C. sphoni* differs from that of *C. fentoni* as the former has a large rachidian tooth on each row, several of the innermost lateral teeth have denticles on both sides of the cusp, and the seminal receptacle is almost as large as the bursa copulatrix (Ortea *et al.* 1992).

Some Caribbean species of *Chromodoris* were described based on limited information on external coloration and anatomy. A group of species with a reticular pattern of yellow

and red pigment includes *Chromodoris binza* Ev. Marcus and Er. Marcus, 1963, *Chromodoris clenchi* (Russell, 1935), and *Chromodoris neona* Er. Marcus, 1955 and was reviewed by Ortea *et al.* (1994). All these species have a large, triangular rachidian tooth on each row of the radula, which is absent in this new species. Additionally, *C. binza* and *C. clenchi* have light rhinophores and branchial leaves with well-marked purple rachises, very different from the whitish rhinophores and branchial leaves with reddish apices of this new species. *Chromodoris neona* has whitish rhinophores and branchial leaves with dark purple apices, but the body color is pale blue with a conspicuous network of bright yellow lines on the dorsum. See Valdés *et al.* (2006) for photographs of these species.

Other Caribbean species that possess a large, triangular rachidian tooth on each row of the radula and are thus easily distinguishable from this new species are *Chromodoris aila* Er. Marcus, 1961, *Chromodoris dictya* Er. Marcus and Ev. Marcus, 1970, and *Chromodoris ponga* Er. Marcus and Ev. Marcus, 1970 (Marcus 1961, Marcus and Marcus 1970). *Chromodoris aila* was described as red with blue and yellowish marks, and *C. ponga* as having a red notum surrounded by a broad white margin, whereas the original description of *C. dictya* contains no information on the color of the live animal.

Chromodoris perola Ev. Marcus, 1976 is a Caribbean species lacking rachidian teeth. The live animals were described by Marcus (1976) as having a

reddish-white mantle surface, with a central row of dark, round, red spots, two lateral rows of elongated red spots, and a deep orange line around the mantle margin. The branchial leaves are opaque, and the rhinophores violet. The anatomy of *C. perola*, described by Marcus (1976), shows several important differences with that of *C. fentoni*. Although the radular morphology of these two species is similar, the jaw rodlets of *C. perola* are short and can be mono, bi, or tricuspid (Marcus 1976: fig. 25). According to the description by Marcus (1976: fig. 27) in the reproductive system of *C. perola* the seminal receptacle opens into the vagina, a short distance

from the vaginal insertion into the bursa copulatrix, and at the same point where the uterine duct opens, with all these three ducts forming a “x” pattern. This is very different from *C. fentoni* in which the vagina opens into the bursa copulatrix at some distance from where both the seminal receptacle and the uterine duct connect directly to the bursa copulatrix. *Chromodoris perola* has not been collected again since its original description and its identity cannot be verified.

More recently, two additional reddish species of *Chromodoris* were described in the Caribbean. *Chromodoris grahami* Thompson, 1980, originally described from Jamaica, is characterized by having a salmon-pink body with bright red spots and dark brown rhinophores (Thompson 1980). *Chromodoris regalis* Ortea, Caballer, and Moro, 2001 is also salmon pink with numerous white spots (Ortea *et al.* 2001). See Valdés *et al.* (2006) for photographs of these species; both are clearly distinguishable from *C. fentoni*.

Eastern Atlantic species of *Chromodoris* with a similar coloration include *Chromodoris luteorosea* (von Rapp, 1827), *Chromodoris luteopunctata* (Gantès, 1962), and *Chromodoris rolani* Ortea, 1988. All these species have a pinkish background color with yellow-orange spots on the dorsum (see Ortea 1988, Cervera *et al.* 1989, Ortea and Valdés 1992), but also have triangular rachidian teeth, which are absent in *C. fentoni*.

Glossodoris punctilucens Bergh, 1890
(Figs. 1C, 4-5)

Chromodoris punctilucens Bergh, 1890: 162-165, pl. 1, figs. 4-10.

Material examined

Off Pinellas County, Florida (28.11750°N, 82.91250°W), 21 June 2009, 9 m depth, 1 specimen 34 mm preserved length, leg. N. Sheridan (FSBC I 67201). Off Pinellas County, Florida (28.19200°N, 83.00770°W), 28 January 2010, 11 m depth, 2 specimens 14 and 15 mm preserved length, leg. D. Fenton (FSBC I 68012).

External morphology

The body is dark brown to black with a number of small white dots and larger yellow spots distributed all over the dorsum (Fig. 1C). Both white dots and yellow spots are situated at the tip of dorsal tubercles. The tubercles vary from small and conical to larger and stalked. The mantle margin is surrounded by a translucent line, followed by a thinner, irregular black line, a broader yellow line and a black-pigmented irregular area. The rest of the mantle margin bears large, oval, discrete, white mantle glands and appears blue probably because of the effect of the white mantle glands under the black epithelium.

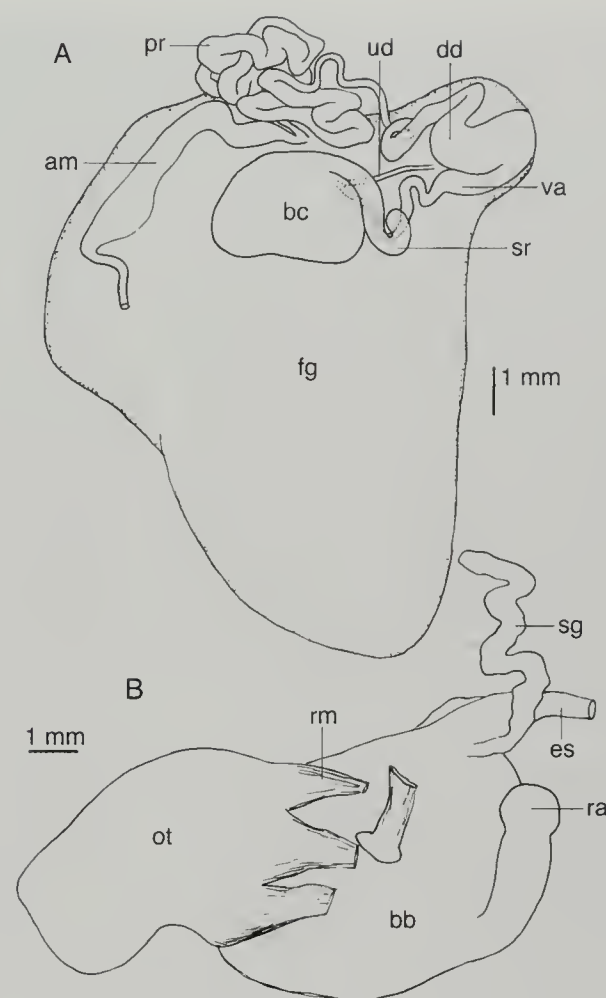


Figure 4. Drawings of the anatomy of *Glossodoris punctilucens* (FSBC I 67201). A, Reproductive system; B, Anterior portion of the digestive system. Abbreviations: am, ampulla; bb, buccal bulb; bc, bursa copulatrix; dd, deferent duct; es, esophagus; fg, female gland complex; ot, oral tube; pr, prostate; ra, radular sac; rm, retractor muscle; sg, salivary gland; sr, seminal receptacle; ud, uterine duct; va, vagina.

There are 50 branchial leaves arranged in the typical double spiral of species of *Glossodoris*. The leaves are translucent beige with dark brown spots and opaque white areas. The rhinophores are dark brown to black with bluish-white pigment on the club and a bright orange apex. The sides of the body are dark brown to black with yellow spots. The margin of the foot sole is surrounded by a yellow line.

Reproductive system

The reproductive system contains a long, narrow ampulla that connects to the prostate and the female gland complex (Fig. 4A). The prostate is long and convoluted and narrows into a long, muscular deferent duct that expands into the broad muscular portion of the deferent duct (Fig. 4A). The vagina is long, convoluted and connects directly into the large, irregular bursa copulatrix. The seminal receptacle is very elongate, and also connects directly into the bursa copulatrix next to the vagina insertion. The straight uterine duct leaves

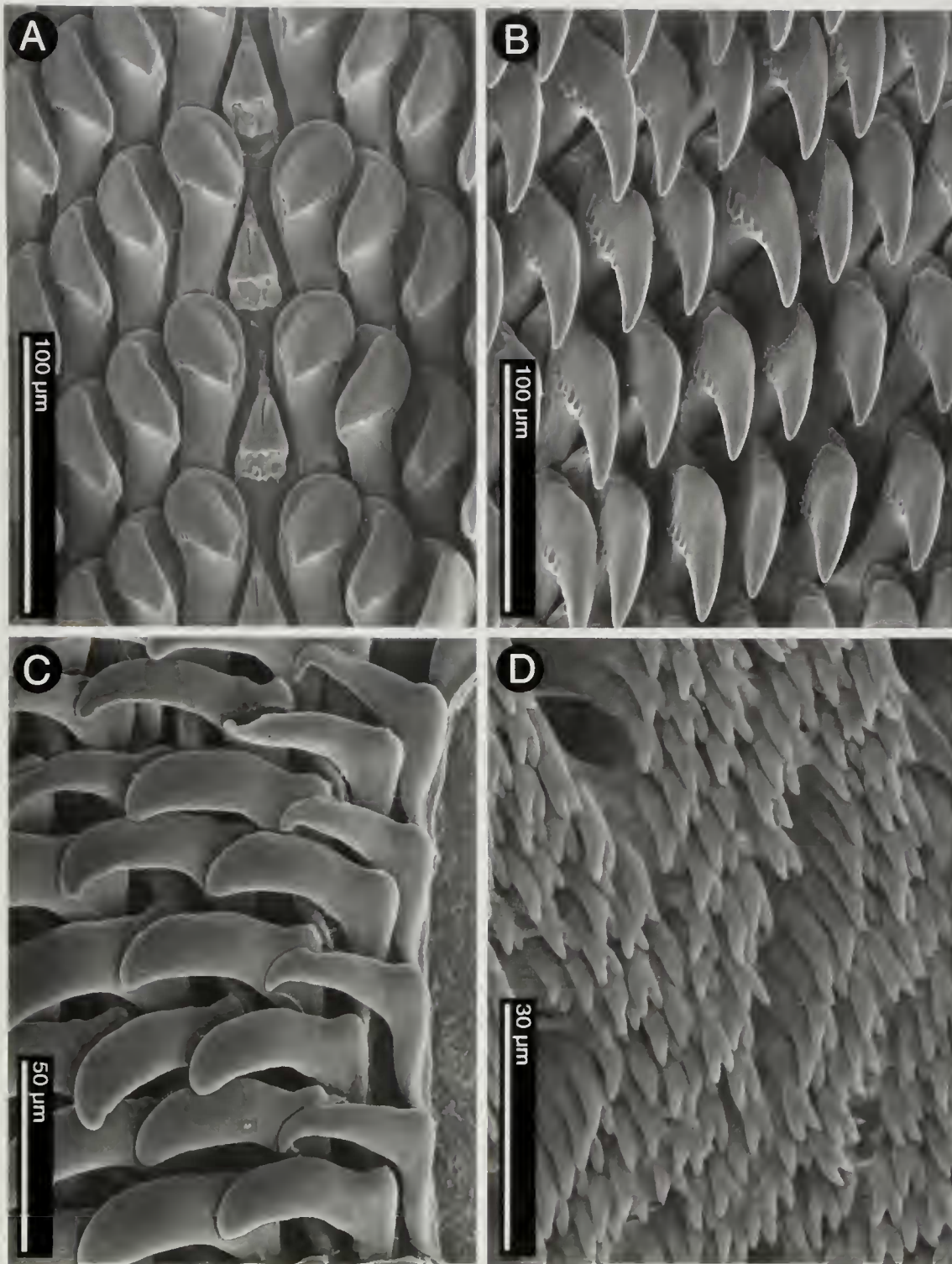


Figure 5. SEM micrographs of the radula and jaws of *Glossodoris punctilucens* (FSBC I 67201). A, Innermost lateral radular teeth; B, Mid-lateral radular teeth; C, Outermost lateral radular teeth; D, Jaw rodlets.

the bursa copulatrix next to the insertion of the seminal receptacle and the vagina, and inserts into the female gland complex, near the opening of the female gland complex.

Digestive system

The buccal bulb is as long as the oral tube (Fig. 4B). The radular formula is $184 \times 51.1.51$ in the single specimen examined. The rachidian teeth are triangular with a thicker apical region divided by a longitudinal notch. The innermost lateral teeth have a shorter cusp than the rest of the laterals

and have 5-7 denticles on both sides of the cusp (Fig. 5A). The mid lateral teeth are hook-shaped with 9-10 denticles on the outer side of the cusp (Fig. 5B). The outer laterals are also hook-shaped and lack denticles (Fig. 5C). The jaw consists of numerous bicuspid (occasionally tricuspid) rodlets (Fig. 5D).

Molecular data

Glossodoris punctilucens is genetically distinct from other species of *Glossodoris* previously studied. The closest species morphologically, *Glossodoris edmundsi* Cervera, García-Gómez, and Ortea, 1989, shows a 7.1% sequence divergence in the COI gene.

Biology

Four of the specimens of *Glossodoris punctilucens* were found on a sponge species tentatively identified as *Ircinia* cf. *campana* (Demospongiae: Dictyoceratida: Irciniidae). Some of the nudibranchs were observed feeding and leaving feeding markings on the sponge, which certainly constitutes their diet. One sponge specimen is deposited at the FWRI collections (FSBC I 069219). *Ircinia campana* belongs to the family Irciniidae (order Dictyoceratida), whereas all known species of *Glossodoris* for which feeding information is available (Rudman and Bergquist 2007) feed on members of the family Thorectidae (order Dictyoceratida). The sponge identification presented here should be regarded as tentative.

Remarks

Chromodoris punctilucens Bergh, 1890 was originally described by Bergh (1890) based on a single specimen collected west of the Dry Tortugas in the Gulf of Mexico at 65 m depth. Subsequently, Valdés *et al.* (2006) published a photograph of another individual collected from an unknown locality in Florida. There are no other published records of this very uncommon species. Subsequent records from the Canary Islands by Odhner (1932) were later identified as a distinct species (see below). Rudman (1984) transferred *C. punctilucens* to *Glossodoris* based on the radular morphology as described by Bergh (1890) and Odhner (1932).

Bergh's (1890) original description and drawings of the radula of *Glossodoris punctilucens* closely match the characteristics of the individual here described. Bergh (1890) described the color of this species as brownish green with numerous yellow and white dots and the mantle margin surrounded by a yellow and a black line. The morphology of the rachidian, inner and outer radular lateral teeth illustrated by Bergh (1890) are virtually identical to those of the specimen here examined.

There are two other Atlantic and one Pacific species similarly colored to *Glossodoris punctilucens*. *Chromodoris ghanensis* Edmunds, 1968 was originally described from Ghana by Edmunds (1968). Edmunds (1968) described this species as having a dark greenish gray or dark bluish gray dorsal color with spots of black, yellow and orange color. The radular morphology is also very similar to that of *G. punctilucens*. Cervera *et al.* (1989) subsequently transferred *C. ghanensis* to *Glossodoris* and described another similar species from the Canary Islands, *Glossodoris edmundsi* Cervera, García-Gómez, and Ortea, 1989 characterized by a grayish-blue dorsum with scattered blackish spots and abundant small orange spots surrounded by smaller yellow ones. Cervera *et al.* (1989) acknowledged the similarities between *G. punctilucens*, *G. ghanensis*, and *G. edmundsi* but distinguished these three species based on anatomical differences, particularly the number of outer radular teeth lacking denticles. Gosliner (1990) re-described specimens of *G. edmundsi* from the Azores and provided the first SEM photographs of the radula, which differs from that of *G. punctilucens* in several details. The rachidian teeth of both Azorean and Madeiran specimens of *G. edmundsi* have a notched, thicker region that occupies almost the entire length of each tooth, whereas in *G. punctilucens* it only occupies the upper 2/3, as observed by Bergh (1890) and the present study. Additionally, the seminal receptacle depicted by Cervera *et al.* (1989) and Gosliner (1990) for *G. edmundsi* is much longer than our observations in *G. punctilucens*. Ortea *et al.* (1996) studied additional specimens from Ghana, Madeira, Azores, and the Canary

Islands, and described their reproductive anatomy and radula. Ortea *et al.* (1996) implicitly considered *G. edmundsi* and *G. ghanensis* as synonyms as they included specimens from Ghana, Madeira, Azores, and the Canary Islands under the same species name (*G. edmundsi*), but at the same time discussed several morphological differences between these two species. We were unable to gather enough information from the literature to determine whether *G. edmundsi* and *G. ghanensis* are synonyms, but morphological and molecular evidence presented here suggests that *G. punctilucens* is a distinct species.

Chromodoris dalli Bergh, 1879 was described by Bergh (1879) stating Puget Sound, Washington, USA, as the type locality. The species was later re-described by Bertsch

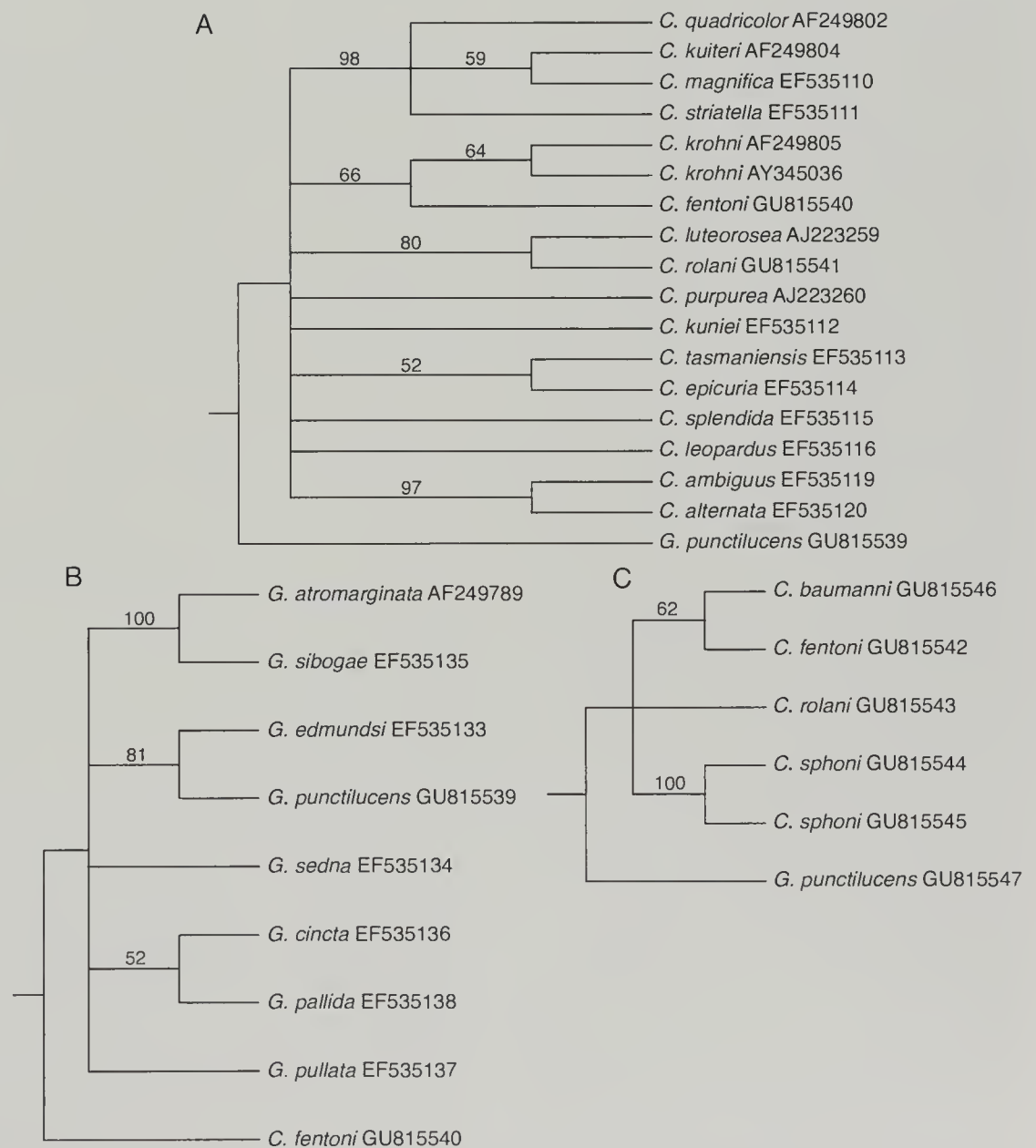


Figure 6. Maximum likelihood bootstrap consensus trees with bootstrap support values. A, COI, *Chromodoris*; B, COI, *Glossodoris*; C, H3, *Chromodoris*. All sequences shown with GenBank accession numbers.

(1978), who suggested that the type material was mislabeled and it was probably collected somewhere in the Gulf of Mexico. Rudman (1984) conclusively transferred this species to *Glossodoris*. *Glossodoris dalli* occurs in the tropical eastern Pacific, from Baja California to the Galápagos Islands (Camacho-García *et al.* 2005). It is characterized by having a lighter color than *G. punctilucens* but both species share numerous tubercles tipped in orange, yellow and white and black spots scattered over the dorsum. The rhinophores and branchial leaves of *G. dalli* are lighter than those of *G. punctilucens* and have orange apices. Ortea *et al.* (1992) described the anatomy and radular morphology of *G. dalli*, which differs from that of *G. punctilucens* in several respects. The seminal receptacle of *G. punctilucens* is curved and inserts directly into the bursa copulatrix, whereas in *G. dalli* it is straight and connects into the vagina, a short distance from the insertion of the vagina into the bursa copulatrix. Ortea *et al.* (1992) described the presence of a vestibular gland in *G. dalli* that was not observed in our specimens of *G. punctilucens*. The radular morphology of *G. dalli* and *G. punctilucens* is very similar, suggesting a close relationship between these two species. Differences between *G. dalli* and *G. edmundsi* include the length of the seminal receptacle, much longer in *G. edmundsi* than in *G. dalli*, and the shape of the rachidian teeth, which have a notched, thicker region that occupies almost the entire length of each tooth in *G. edmundsi*, whereas in *G. dalli*, as in *G. punctilucens*, this thicker region only occupies the upper 2/3 of each tooth.

DISCUSSION

The molecular trees produced in this study (Fig. 6) do not aim to provide a detailed account of the phylogenetic relationships of the two species studied, but just to help, along with the morphological data, to properly characterize the two species. Our goal in providing sequence data along with the description of the new species is to facilitate further work on the systematics and biogeography of *Chromodoris* and test the validity of the new taxon.

For the most part, the COI and H3 bootstrap consensus trees are poorly resolved and most likely would require many more taxa to provide a clear phylogenetic signal; this is however beyond the scope of this paper. The levels of saturation in all the alignments were low and therefore they should not have affected the phylogenetic signal.

Several limited conclusions can be drawn from the phylogenetic trees. The COI tree of the species of *Chromodoris* (Fig. 6A) is poorly resolved and shows very low support for a sister relationship between *Chromodoris fentoni* and *Chromodoris krohni*. However, it is important to note that the sister relationship between two other Atlantic species, *Chromodoris luteorosea* and *Chromodoris rolani*, is well supported. These two very similar species also appear to be distinct. Many other Atlantic and eastern Pacific species need to be included



Figure 7. Maximum likelihood bootstrap consensus tree of species of *Chromodoris* and *Glossodoris* with bootstrap support values. All sequences shown with GenBank accession numbers.

in this analysis in order to say anything meaningful about the relationships within this group. Unfortunately we were unable to amplify COI for potentially closely related species such as *Chromodoris sphoni* and *Glossodoris baumanni*, from which we obtained H3 sequence data, and we had no access to material from other species such as *Chromodoris luteopunctata*.

The *Glossodoris* COI tree (Fig. 6B) show strong support for the sister relationship between *Glossodoris edmundsi* and *Glossodoris punctilucens*, but as mentioned above, the molecular apomorphies present in these two species as well as anatomical differences justify their separation into two different species.

The combined *Chromodoris* + *Glossodoris* tree (Fig. 7) is also poorly resolved and does not recover the basal relationships between most species and clades. However, this tree provides important information on the phylogenetic position of *Chromodoris fentoni* and *Glossodoris punctilucens*. *Chromodoris fentoni* is nested in a clade containing the two eastern Atlantic species *Chromodoris purpurea* and *C. krohni*, which further supports the placement of *C. fentoni* in *Chromodoris*. Additionally, the combined analysis shows strong support for the sister relationship of *G. punctilucens* and *G. edmundsi*, which appear to be very closely related species. Finally the H3 tree (Fig. 6C) is very incomplete and provides no relevant information except for showing divergence between *Chromodoris fentoni* and other similar species.

The combination of molecular and morphological information in this study provides additional support for the taxonomic decisions. This paper also provides information that will be useful in investigating the relationships among other Caribbean and Gulf of Mexico species of chromodorid nudibranchs.

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